THE CHANGE IN VOLUME OF ARBACIA AND ASTERIAS EGGS AT FERTILIZATION.[1]

OTTO GLASER.

In my paper on the initiation of development, Glaser (13), I stated that the eggs of *Arbacia punctulata* and *Asterias forbesii* are smaller in volume after fertilization than before. In the pages that follow I wish to present the evidence for this assertion in some detail, since for the present at least, it must stand alone. *Strongylocentrotus* (*purpuratus?*) shows no recognizable loss of volume according to Loeb ('08) but this result does not contradict mine. The loss depends on several conditions, and in *Asterias* is much greater than in *Arbacia*. This suggests the possibility of forms in which it may easily be too small to measure. The measurements of McClendon ('10), also, do not contradict mine, although they seem to show that the very eggs I worked with, on fertilization lose in a molar solution of dextrose, but remain constant, or even gain a little in sea water. The evidence consists in the determination of the diameters of 19, 20, 10 and 11 eggs respectively. The great variability of the ova of *Arbacia punctulata*, however, proves that reliable results can be gotten from a small number of eggs, only when the identical ones are measured before and after fertilization.

Careful tracings were made at known magnifications with the aid of a camera lucida, and a crow-quill pen, on paper of known weight. On the assumption that the eggs are spheres, and that one is really drawing their great circles, the areas of the outlines obtained in this manner, were first determined by means of a planimeter, and afterwards by carefully cutting them out and weighing the discs. For enlargements of 740 diameters both methods gave identical results, but for the smaller magnifications the method of weighing proved more reliable. For this reason, in the comparisons of fertilized with unfertilized eggs, only data

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obtained by the latter method are reported, whereas the variability curves are based on factored measurements by the planimeter. The same curves were first constructed from the original measurements.

On the Constancy in Volume of the Eggs of a Single Female.

In Table I. are given the diameters of 280 different eggs derived from one female, measured in groups of ten, at three distinct periods after removal from the ovaries. The results show not only a remarkable constancy for the ova of a single individual, when grouped in this manner, but what is of even more immediate importance, they show that in four hours, the eggs do not undergo any change in volume discoverable by the methods employed.

Table I.

Female B.

10 eggs, 4.35 P.M. 81.4 micra.	5.40 P.M. 82.6 micra.	8.35 P.M. 79.6 micra.
10 eggs, 4.35 P.M. 80.0 micra.	5.40 P.M. 80.8 micra.	8.35 P.M. 81.6 micra.
10 eggs, 4.35 P.M. 81.2 micra.	5.40 P.M. 80.8 micra.	8.35 P.M. 79.6 micra.
10 eggs, 4.35 P.M. 80.6 micra.	5.40 P.M. 79.4 micra.	8.35 P.M. 79.0 micra.
10 eggs, 4.35 P.M. 82.4 micra.	5.40 P.M. 78.6 micra.	8.35 P.M. 81.0 micra.
10 eggs, 4.35 P.M. 80.6 micra.	5.40 P.M. 81.6 micra.	8.35 P.M. 82.4 micra.
10 eggs, 4.35 P.M. 78.8 micra.	5.40 P.M. 81.2 micra.	8.35 P.M. 82.2 micra.
10 eggs, 4.35 P.M. 81.4 micra.	5.40 P.M. 80.8 micra.	8.35 P.M. 81.0 micra.
10 eggs, 4.35 P.M. 78.2 micra.	5.40 P.M. 77.4 micra.	8.35 P.M. 80.0 micra.
10 eggs, 4.35 P.M. 83.6 micra.	5.40 P.M. micra.	8.35 P.M. micra.
Average 80.8 micra.	Average 80.4 micra.	Average 80.6 micra.

On the Volume of the Eggs of Different Females.

To what extent the average volume of the ova of different females is constant, I am unable to say, since scarcely anything much less than one hundred eggs from each would suffice to decide the question. One would not expect great constancy however unless the numerous factors entering into the result were identical. In the one case in which I have the requisite number of measurements for such comparison, the average diameter is the same as that found for female B.

Table II.

Female C.

10 eggs	80.4 diam.
10 eggs	82.2 diam.
10 eggs	80.2 diam.
10 eggs	79.6 diam.
10 eggs	81.6 diam.
10 eggs	79.0 diam.
10 eggs	77.8 diam.
10 eggs	79.2 diam.
10 eggs	79.2 diam.
Average	80.0 diam.

The Diameters of Individual Eggs.

In the two preceding tables the measurements are given for groups of ten eggs each. Comparison of the various sets indicates considerable variability, but the true extent of this can only be realized when a large number of ova is treated individually. This was done with 100 eggs of female B and 100 of female C. The results are given in Tables III. and IV.

Table III.

Female B, Individual Eggs.

Of 100 eggs	1 had a diameter of	49.8 micra.	
Of 100 eggs	1 had a diameter of	58.8 micra.	Average of 3 = 57.1
Of 100 eggs	1 had a diameter of	61.6 micra.	
Of 100 eggs	1 had a diameter of	64.2 micra.	
Of 100 eggs	1 had a diameter of	65.4 micra.	Average of 3 = 65.5
Of 100 eggs	1 had a diameter of	66.8 micra.	
Of 100 eggs	2 had a diameter of	68.0 micra.	
Of 100 eggs	4 had a diameter of	70.6 micra.	Average of 11 = 70.8
Of 100 eggs	5 had a diameter of	72.0 micra.	
Of 100 eggs	5 had a diameter of	73.2 micra.	
Of 100 eggs	3 had a diameter of	74.6 micra.	Average of 16 = 74.2
Of 100 eggs	8 had a diameter of	76.0 micra.	
Of 100 eggs	3 had a diameter of	77.2 micra.	
Of 100 eggs	5 had a diameter of	79.6 micra.	Average of 15 = 79.2
Of 100 eggs	7 had a diameter of	79.8 micra.	
Of 100 eggs	8 had a diameter of	81.2 micra.	
Of 100 eggs	2 had a diameter of	82.4 micra.	Average of 20 = 82.6
Of 100 eggs	10 had a diameter of	83.8 micra.	
Of 100 eggs	7 had a diameter of	85.0 micra.	
Of 100 eggs	4 had a diameter of	86.4 micra.	Average of 14 = 84.5
Of 100 eggs	3 had a diameter of	87.6 micra.	
Of 100 eggs	6 had a diameter of	89.0 micra.	

TABLE III. *Continued.*

FEMALE B. INDIVIDUAL EGGS.

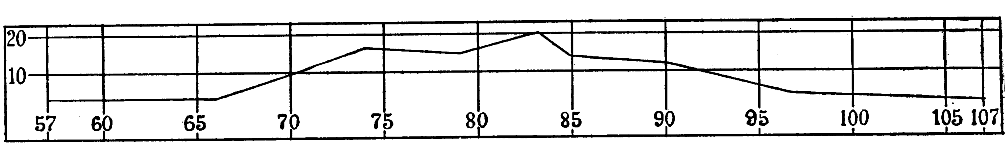
Of 100 eggs	3 had a diameter of 90.4 micra.	Average of 12 = 90.0
Of 100 eggs	3 had a diameter of 91.6 micra.	
Of 100 eggs	2 had a diameter of 94.2 micra.	
Of 100 eggs	1 had a diameter of 96.8 micra.	Average of 4 = 96.5
Of 100 eggs	1 had a diameter of 100.8 micra.	
Of 100 eggs	1 had a diameter of 102.0 micra.	
Of 100 eggs	1 had a diameter of 112.6 micra.	Average of 2 = 107.3

TABLE IV.

FEMALE C, INDIVIDUAL EGGS.

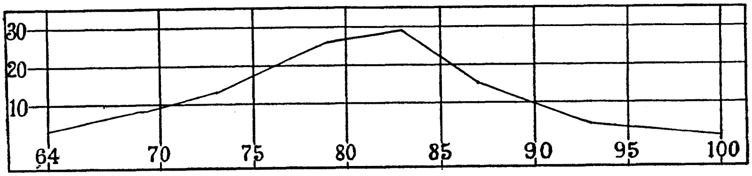
Of 100 eggs	1 had a diameter of 59.4 micra.	
Of 100 eggs	1 had a diameter of 65.2 micra.	Average of 3 = 63.7
Of 100 eggs	1 had a diameter of 66.6 micra.	
Of 100 eggs	2 had a diameter of 68.0 micra.	
Of 100 eggs	5 had a diameter of 69.6 micra.	Average of 8 = 69.4
Of 100 eggs	1 had a diameter of 71.0 micra.	
Of 100 eggs	5 had a diameter of 72.2 micra.	
Of 100 eggs	4 had a diameter of 73.8 micra.	Average of 13 = 72.9
Of 100 eggs	4 had a diameter of 75.4 micra.	
Of 100 eggs	6 had a diameter of 76.8 micra.	
Of 100 eggs	9 had a diameter of 78.2 micra.	Average of 26 = 78.5
Of 100 eggs	11 had a diameter of 79.6 micra.	
Of 100 eggs	8 had a diameter of 81.0 micra.	
Of 100 eggs	11 had a diameter of 82.6 micra.	Average of 29 = 82.6
Of 100 eggs	10 had a diameter of 83.8 micra.	
Of 100 eggs	7 had a diameter of 85.6 micra.	
Of 100 eggs	4 had a diameter of 87.0 micra.	Average of 15 = 86.7
Of 100 eggs	4 had a diameter of 88.4 micra.	
Of 100 eggs	2 had a diameter of 89.8 micra.	
Of 100 eggs	1 had a diameter of 94.2 micra.	Average of 4 = 92.4
Of 100 eggs	1 had a diameter of 95.6 micra.	
Of 100 eggs	1 had a diameter of 100.0 micra.	Average of 1 = 100.0

If one treats the diameters and eggs in groups of three, and plots the latter on the ordinate and the former on the abscissa the following curve emerges for female B.



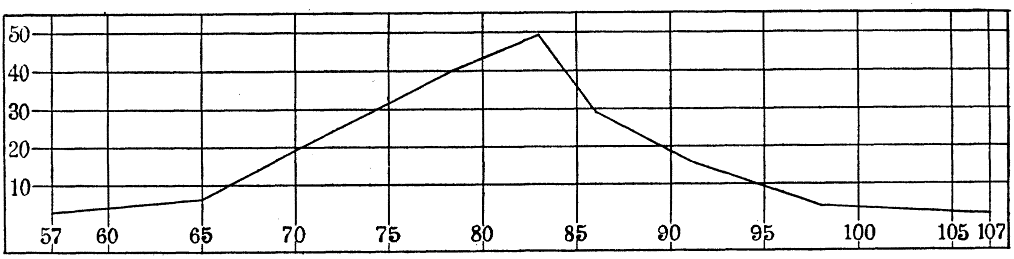
CURVE B. Diameters of 100 eggs from one specimen of *Arbacia punctulata*.

The data from female C, treated similarly, give curve C.



CURVE C. Diameters of 100 eggs from one specimen of *Arbacia punctulata*.

Since the diameters for females B and C on the average are equal, the eggs of these two may be considered as homogeneous material. If this is done, then after the usual statistical simplifications,[1] an almost symmetrical curve results.



CURVE BC. A composite of the diameters of 200 eggs from two specimens of *Arbacia punctulata*.

COMPARISON OF UNFERTILIZED AND FERTILIZED OVA.

The variability exhibited by these eggs and the differences in range between different females, show clearly that if one wishes to make trustworthy comparisons between the volumes of unfertilized and fertilized ova, it is either necessary to use a large number of each kind from a single female or to deal with a smaller number and measure the identical eggs before and after fertilization.

Before adopting the second alternative, I made a series of tracings of unfertilized and fertilized ova to see whether measureable differences really existed. The results, averaged, were as follows:

Unfertilized eggs;	77 measurements;	average diameter, 74.1 micra.
Fertilized eggs;	91 measurements;	average diameter, 70.7 micra.
		Loss 3.4 micra.

[1] The eggs are treated in sets of three, and average diameters not more than three micra apart are considered as belonging to the same group.

This result, further discussion of which is unnecessary, encouraged me to undertake a more careful study of a few eggs. Although involving more labor, this alternative was chosen in preference to the "wholesale" method, because the behavior of individual eggs could be definitely recorded. The probable advantage of this was indicated by the discovery by Glaser ('13) that the number of sperm used in insemination plays a rôle in the result. Accordingly "wholesale" measurements, which are satisfactory enough to decide whether or not a determinable change in volume occurs, have the disadvantage of covering up the fact that the loss is by no means a constant quantity.

In making these particular measurements, special precautions were taken. The eggs were traced one at a time, no other eggs were on the slide, and the focus before and after fertilization remained unchanged. This was not easy to accomplish, for the sperm are apt to set the egg in motion and change its position. Whenever this occurred, the attempt to secure the second measurement was abandoned. Naturally only a small number of cases could be collected under these conditions.

In order to fulfill the aforesaid requirements, and keep the magnification of the tracings absolutely constant, it was necessary to use small quantities of sperm. This however introduced other difficulties. The appearance of the fertilization membrane is the only convenient index of impregnation, but the membrane does not appear unless the spermatozoa are present in sufficient numbers. As a consequence, I often failed to observe the only available indication of fertilization.

While the results which I have seem to me to point in but one direction, the changes in volumes registered are in most cases probably minimal, for they depend on the amount of surface alteration, and this in turn is, within limits, a function of the number of sperm involved. Indeed with great excesses of sperm I have not infrequently observed a destructive alteration of the surface of the egg. This is more easily observable in *Asterias* than in *Arbacia*. It appears likely therefore that normally, or perhaps better, frequently, greater changes in volume occur than I have recorded.

The results on individual eggs dealt with singly in the manner described are given in the subjoined table.

Table V.

Individual Eggs Before and After Fertilization.

Female.	Egg.	Measurements.	Before.	Measurements.	After.	Change in diameter.
D1	1	3	81.2	2	75.4	−5.8
D1	2	3	80.6	3	68.8	−11.8
D1	3	3	73.4	3	65.4	−8.0
D1	4	3	78.8	3	72.8	−6.0
D1	5	3	74.6	3	68.4	−6.2
D2	1	4	70.0	3	66.6	−3.4
D2	2	4	71.2	4	67.2	−4.0
D2	3*	3	78.0	4	78.2	+0.2*
D2	4	4	75.2	3	73.4	−1.8
D2	5	3	78.0	4	75.2	−2.8
E	1	3	70.0	3	68.2	−1.8
E	2*	4	65.2	2	65.8	+0.6*
E	3	3	72.2	4	65.6	−6.6
E	4	3	72.2	4	69.4	−2.8
E	5	3	68.8	4	67.8	−1.0

With the exception of the starred eggs, D2 3, and E 2, which within the limits of error may easily be considered constant, all the eggs showed a noticeable decrease.

The fact brought out by these measurements constitutes an additional support for the conception that an increase in the permeability of the egg accompanies fertilization. With such increase, other conditions remaining constant, one would expect a change in volume. The fact that unfertilized eggs, under certain conditions, may remain unaltered for hours, whereas there is a noticeable decrease in their diameters immediately after fertilization, strongly emphasizes the change that takes place.

Comparison of Unfertilized and Fertilized Ova of *Asterias*.

The results obtained in the case of *Arbacia* are more strikingly illustrated by a control observation made on the ova of *Asterias forbesii*. The measurements were made on eggs which had been removed from the ovaries and allowed to stand one hour in sea water in order to complete maturation. Tracings were made of two small groups from two females and these groups were then inseminated. It is impossible to identify individual eggs in this case, but this does not invalidate the result, since no eggs were either added or removed upon the addition of the sperm. Seventeen eggs derived from one female, and eight from the other

were used, and the same eggs were traced after fertilization that had been traced before. The result was:

Female 1; 17 eggs	Diameter before fertilization, 103.6 micra. Diameter after fertilization, 85.2 micra.	Loss 18.4 micra.
Female 2: 8 eggs	Diameter before fertilization, 105.0 micra. Diameter after fertilization, 94.0 micra.	Loss 11.0 micra.

These values cannot be considered standard figures any more than those found in the case of the sea urchin, yet they seem to indicate with certainty that the egg of the starfish also loses in volume on fertilization. In fact the loss recorded is much greater than that found for *Arbacia* eggs. A portion of this difference may be due to unequal amounts of sperm used in the two cases, but the entire variation can hardly be accounted for on this basis. Normally the perivitelline space of fertilized *Asterias* eggs is proportionately larger than in *Arbacia*. If the space in question comes about as Loeb ('08) has contended, when a colloidal substance secreted by the egg absorbs water, then on the assumption that this material has essentially the same properties in *Arbacia* and in *Asterias*, the egg having the greater vitelline space should secrete the greater quantity, and therefore decrease correspondingly more in volume. That the *Asterias* eggs measured lost more on the average than the *Arbacia* treated either en masse, or as individuals, can be seen by a glance at the tables. One is led to suspect from these considerations that the surface of the *Asterias* egg is more easily modifiable than that of the *Arbacia* ovum. This is in harmony with the well known experience that artificial parthenogenesis can be induced in *Asterias* by a greater variety of methods than in *Arbacia*.

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